METHOD FOR PRODUCING RECOMBINANT PROTEINS WITH A CONSTANT CONTENT OF PCO_2 IN THE MEDIUM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the National Stage of International Application No. PCT/EP2009/001742, filed Mar. 11, 2009, which claims benefit to German Application No. 10 2008 013 899.1 filed on Mar. 12, 2008. The contents of the applications cited above are incorporated by reference in their entirety.

FIELD OF THE INVENTION

Background of the Invention

The present invention relates to a method for the recombinant production of a polypeptide in a eukaryotic host cell modified in the citrate cycle, wherein the cell is cultured in a medium with a content of dissolved CO_2 (pCO_2) which is maintained at a constant value in the range of 10% to 20%.

The ton-scale production of therapeutic proteins for specific therapies implies new requirements regarding expression and production systems. The main expression systems used are, amongst others, animal cell culture systems. The capability of animal cells to correctly fold and post-translationally modify proteins is, above all, a requirement for clinical application in humans. At present, almost 70% of all recombinant proteins are produced in animal cells in the pharmaceutical industry, most of these in CHO cells (*Chinese Hamster Ovary cells*) (Wurm, 2004). In comparison to microbiological systems, however, animal cell culture processes are characterized by longer generation time and lower final cell density in fermentations. Thus, product titres and space-time yields are lower than in microbiological processes. One possibility to compensate this disadvantage is metabolic engineering, i.e. controlling cell growth and minimizing apoptosis, programmed cell death, by genetic modification of the producing cells. In addition to genetic approaches, culture control strategies prove to be suitable optimization approaches the potential of which is often underestimated. It is, for example, possible to efficiently influence glycosylation, carbon metabolism, cell growth and cell death by using procedural monitoring and control strategies as well as the composition of culture media.

Thus, procedural development aims not only at the development of metabolically optimized cell lines but also at maximum exploitation of the potential of an existing cell line by optimum medium conditions and optimum process control. Apart from process parameters easily controllable such as oxygen content and temperature, more complex influence factors such as the content of dissolved carbon dioxide are taken into consideration for process control. In animal cell culture processes, CO_2 accumulates as final product in physically dissolved form and chemically dissociated as hydrogencarbonate in aqueous culture media. Today, the development towards high cell density processes using animal cells in fed batch processes results, in conjunction with industrially used large-volume fermenters and hydrostatic pressures prevailing therein, i.e. in CO_2 partial pressures of 150-200 mm Hg which, consequently, are five times higher than the cell physiological values of 31-54 mm Hg. For example, for the provision of polypeptides (cytokines) produced by recombinant CHO cells, said cells were cultivated at 37° C. and 5% CO_2 (36 mm Hg pCO_2) in order to express the recombinant cytokines (U.S. Pat. No. 6,406,888B1). Fogolin et al., Journal of Biotechnology, 2004, 109, 179-191 shows that the genetic modified cell line CHO-K1-hGM-CSF expresses a recombinant yeast pyruvate carboxylase (PYC2) at 37° C. and 5% CO_2. The results of this study revealed that the expression of PYC2 and a reduced culture temperature have an additive effect on the cell specific productivity of the genetic modified cell line CHO-K1-hGM-CSF. Moreover, the effects of elevated pCO_2, osmolarity on the growth rate and specific human tPA production rate of a recombinant CHO cell line have been studied by Kimura and Miller, Biotechnology and Bioengineering, 1996, 52, 152-160. The media used for the experiments in this study contain 36 mm Hg pCO_2 (5% CO_2), 140, 195, 250 mm Hg pCO_2. However, these authors were of the opinion that the highest recombinant protein production rate can be achieved at 37° C. and 36 mm Hg pCO_2 (5% CO_2). Adverse effects on growth and productivity for hybridomas, NS0, CHO, BHK and insect cells have been reported for such high pCO_2 concentrations. In industrial large-scale fermenters, the accumulation of CO_2 is a restrictive factor. The desorption of dissolved CO_2 from the culture medium is a challenge for process engineering. Thus, the oxygen supply for the cultured cells has to be provided. Correcting variables for enhancing the oxygen transfer into the liquid phase are, for example, stirrer velocity and volumetric gas flow. However, these cannot be freely modified due to the partially cell disrupting shear stress and foaming.

BRIEF SUMMARY OF THE INVENTION

Thus, the technical problem underlying the present invention is to provide an optimized method for the recombinant production of proteins in eukaryotic host cells which overcomes the disadvantages described above, i.e. which throughout provides an optimum content of physically dissolved CO_2 in the medium.

The solution of this technical problem is provided by the embodiments characterized in the patent claims. The strategy leading to the present invention was based on the approach of controlling the set value of pCO_2 with simultaneous control of dissolved oxygen, pH value and overpressure in the reactor. This rational approach of the decoupled control of as many parameters as possible associated with pCO_2-related difficulties resulted in the surprising finding that the yield of recombinantly produced protein unexpectedly increased with a pCO_2 value maintained constant in the range of 10% to 20% of dissolved CO_2. Moreover, the control of the pCO_2 concentrations over the entire process of fermentation had a positive effect on culture viability and allowed for a prolonged stationary phase of high cell density. The respective results of the strategy resulting in the present invention when applied to the culture of animal cells are summarized below:

(a) pCO_2 Control

Based on an in situ sterilisable pCO_2 probe, a pCO_2 controller was developed for the studies which would meet industrial requirements and which was implemented successfully in development fermentation. With simultaneous, independent control of pO_2 and pH, this pCO_2 controller allows both, pCO_2-static culture and the generation of set value profiles for pCO_2. Applications in fed-batch mode and chemostat mode were established successfully on the 1 L and 10 L scale. The control range is 1.5-25.0% pCO_2. Thus, for the parameter CO_2, it is possible to deal with problems in industry on a small scale.

(b) Overpressure Control

An overpressure valve was developed for scales 1 L (up to 150 mbar overpressure) and 10 L (up to 1000 bar overpressure) which may be used independently from the controls for